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Quality Stability and Antioxidant Activity of Red Ginseng Stored for Long Periods

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Abstract

Samples of red ginseng, which had been manufactured and packaged by the Korea Monopoly Corporation, were stored at ambient temperatures (12-28°C) and humidities (55-68%) for one to nine years to examine their overall quality stability and, in particular, antioxidant activity. The approximate compositions, contents of various solvent extracts, and TLC and HPLC patterns of ginsenosides in the samples which are otherwise susceptible to oxidation, were stable as judged by the changes of the TLC and GLC patterns of the lipids and fatty acids. It was also found that polyunsaturated fatty acids such as linoleic (C18 : 2) and linolenic acid (C18 : 3) present in the samples had been very stable during the long storage periods. The intensity of the brown color of the red ginseng samples increased significantly with storage time. The pH of the aqueous extracts of the samples also increased slightly during storage. The coloration changes seem to indicate that extensive browning reactions had occurred during storage. The reducing powers of aqueous and ethanol extracts and antioxidant activity of ethyl acetate extracts also increased with storage time. The increase in reducing powers and antioxidant activity appeared to be directly attributable to the increased amounts of non-enzymatic browning reaction products formed progressively during the long storage periods. Therefore, it seems that those antioxidative compounds, which will be progressively formed in red ginseng through non-enzymatic browning reactions during the manufacturing process and long-term storage, will not only contribute to their overall quality stability but also have some significant relationship with their anti-aging pharmacological effects.

Introduction

Since about the tenth century, A.D., red ginseng has been manufactured in Korea and exported to other countries. The raw ginseng roots have been steamed and dried in order to improve their pharmacological and storage efficiency; this manufacturing procedure has remained basically unchanged. The market cost in the same grade of Korean red ginseng generally varies depending on the length of its storage period. The quality of Korean red ginseng is known to be stable with time, and some contend that increased storage time may actually enhance its pharmacological effects.

The purpose of this study was to determine whether or not these claims had any scientific basis.

Materials and methods

Red ginsengs : Six-year-old raw ginsengs were harvested in September or October every year from 1977 to 1986, steamed and dried by heating in Korea Ginseng Factory of Korea Monopoly Corporation to make red ginseng. The red ginsengs had been stored at the temperature of 12-28°C and the RH of 55-68%, and were sampled for the study in February 1987 and pulverized to 80 mesh.

Reagents : Standards of fatty acid methyl esters and other lipids were purchased from Nu Check Prep Inc. (Elysian, MN, USA). BF_3-methanol was from Sigma Chemical Company (St. Louis, Mo. USA). Thiobarbituric acid (TBA) was from Fluka (Switzerland). Silica gel plate and HPLC solvents were from E. Merck Co. (Germany). Other color reagents and solvents were guaranteed grade.

Appearances and panel tests : Packaging states and appearances of the samples were observed by naked eye. Panel test was carried out to examine odor and taste of the samples by comparing them with the red ginseng manufactured in 1986 as a control. Panel test was carried out by adding 100 ml of hot water to 2g of samples.

Approximate composition : Moisture, ash, crude fat, crude protein, crude fiber and total sugar contents were analyzed by A.O.A.C. methods[1]. Each reducing sugar was analyzed by HPLC[2,3] to convert into total reducing sugar.

Yields of 50% ethyl alcohol and aqueous extract : 50% ethyl alcohol and aqueous extracts were measured as described in the general experimental procedure of Korean pharmacopeia(Ⅳ). The yields of the extracts were expressed as dry basis(%).

Contents of saponins : According to the method of Ando et al.[4], saponin fraction was separated by the usual water-saturated butanol method. The contents of crude saponin, total saponin and ginsenosides were determined by gravimetric method[4], Vanillin-H_2SO_4 colorimetry[5-8] and HPLC[3,9], respectively.

Thin layer chromatography (TLC) of saponins : TLC of the saponin was performed on plate pre-coated with 0.25mm layer of silica gel. Lower layer of $CHCl_3$/n-BuOH/MeOH/H_2O (20 : 40 : 15 : 20, v/v)[10] was used for developing the chromatography. The spots were visualized by spraying with 30% H_2SO_4 and heating the plate at 110°C for 15 min.

Analysis of fatty acids and lipids : Lipids were extracted from powdered samples with ethyl ether by soxhlet extraction method[11]. Lipid components were chromatographed by TLC on silica gel plate with petroleum ether/ ethyl ether/ acetic acid (80 : 20 : 1, v/v) and visualized with 30% H_2SO_4. The content of each lipid component was analyzed by TLC scanner[3,11]. The methyl esters of fatty acids were prepared by the method of Metcalf[12] using BF_3-methanol. The fatty acid methyl esters were analyzed by GLC using a 30m x 0.32mm I.D. SP-2340 fused silica capillary column (Supelco INC., Bellefonte, PA). Column temperature was held at 150°C for 5 min. and then increased to 200°C by 4°C/min. Detector and carrier gas were FID and nitrogen, respectively.

Color intensity : The color intensities of the samples pulverized to 115 mesh were measured with color difference meter according to CIE standard color system and Hunter color system method described in ASTM committee method[13]. The brown color intensities of

water extracts were measured at 490nm by spectrophotometer.

pH value : Powdered red ginseng (2.0g) was extracted with 100ml of water at room temperature for 24 hrs. and then pH of the solution was measured.

Free sugars and free amino acids : Free sugars and free amino acids were analyzed by HPLC[2,3] and amino acid autoanalyzer[3], respectively.

Free radical quenching activity : 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was dissolved in ethanol and diluted appropriately until its absorbance(A_{525}) was shown to be 0.97. With 2ml of DPPH solution, 2ml of ginseng extract solution (100ml/g) was mixed and the absorbance decrease during ten minutes at 525nm was measured as a free radical quenching activity[3,14,15]

Antioxidant activity : Antioxidant activities of the samples were estimated by TBA value. TBA value was obtained by the method described by Mitsuda et al.[16] and Choi[3]. The ethyl acetate fraction[3] containing ethyl ether fraction of red ginseng, which showed strong anti-oxidant activities, was added to linoleic acid in 0.1M phosphate buffer. After having kept standing for 10 hours, absorbance was measured at 532nm to give TBA value (A_{532} x 100).

Inhibitory effect against lipid hydroperoxide

a) Preparation of hepatic microsomal fraction : The liver was isolated from male Sprague-Dawley rat, weighing 180-200g. After mincing, the piece of liver was homogenized in 4 vol. of homogenizing solution (150mM KCl, 50mM Tris-HCl, pH 7.4). The homogenate was centrifuged at 12,000g for 20 min. The resulting supernatant was centrifuged at 100,000xg for 60 min., and the microsomal fraction resuspended in homogenizing solution to a final concentration of 10mg of protein per ml. Protein was determined by the method of Lowry et al.[17] using bovine serum albumin as a standard.

b) Lipid peroxidation assay in hepatic microsome : In enzymatic Paraquat/NADPH system[18], microsome (1mg protein/ml) was incubated for 1 hr. at 37°C in 50mM Tris-HCl buffer (pH 7.5) with 10μM Paraquat and 0.5 mM NADPH. On the other hand, in non-enzymatic Cu^{++}/H_2O_2 system[19], the microsome was treated as the above same method with 50μM $CuSO_4$ and 5mM H_2O_2.

And also 50μg/ml of the ethyl acetate extract of red ginseng was added, and the total volume of the reaction mixture was 2.0ml. The amount of MDA, lipid hydroperoxide, was measured by TBA method (A_{533})

Results and discussion

Appearances and panel tests : Samples of red ginseng were stored at ambient temperatures and humidities (12-28°C and 55-68 percent) for one to nine years to examine external appearance of packagings and sensory evaluation of red ginsengs. As shown in Table 1, packagings and red ginsengs remained unchanged for long storage periods.

Approximate composition : Moisture contents of red ginsengs, whose contents were 9.0-10.8%, showed a little decrease for long storage. Ash was 3.5 to 4.1%, crude fat 1.1 to 1.2%, crude protein 12.3 to 14.9%, crude fiber 5.0 to 7.1%, and total sugar 60.4 to 64.6%.

These data indicate that the differences in the contents of approximate compositions were very slight. But reducing sugar was 13.21% in the control and decreased to 7.79% in red ginseng stored for 9 years. It suggests that non-enzymatic browning reaction resulted in the decrease of reducing sugar during storage.

Yield of water solvent extracts : As shown in Table 3, the yields of water and 50% ethanol extracts from the red ginseng sample were unchanged, but yields of ethyl acetate and methylene chloride extracts increased slightly with storage time.

Comparison of saponin contents and TLC profiles : Crude and total saponins were determined by gravimetric method[4] and vanillin-H_2SO_4 colorimetry[5-8], respectively, which showed no changes during long storage as 4.1 to 4.3% and 2.7 to 3.1%. The ratio of PD to PT saponins as well as each ginsenoside contents analyzed by HPLC[3,9] remained unchanged during storage. TLC profile (Fig. 1) shows that red ginsengs manufactured and stored through years have the same kinds and patterns of saponins.

Percent composition of lipids : Fig. 2 and Table 5 show TLC chromatogram of crude lipids extracted with ethyl ether and percent composition of lipid components

Table 1. External appearance and sensory evaluation of the red ginsengs stored for long periods

Item \ Stored year	0	1	2	3	4	5	6	7	8	9
Manufactured year	1986	1985	1984	1983	1982	1981	1980	1979	1978	1977
Can packaging										
Printing	G*	G	G	G	G	G	G	G	G	G
Seaming	G	G	G	G	G	G	G	G	G	G
Corrosion	–**	–	–	–	–	–	–	–	–	–
Adhesion	G	G	G	G	G	G	G	G	G	G
Pin hole	–	–	–	–	–	–	–	–	–	–
Red ginseng										
Growth of mold	–	–	–	–	–	–	–	–	–	–
Sensory evaluation	G	G	G	G	G	G	G	G	G	G

* : good, ** : not detected

Table 2. Percent proximate composition of the red ginsengs for long periods

(Unit ; % of dry basis)

Item \ Stored year	0	1	2	3	4	5	6	7	8	9
Moisture	10.85	10.43	10.26	10.61	10.14	10.30	10.18	9.08	10.14	9.87
Ash	3.96	4.11	3.83	3.90	3.82	3.66	3.75	3.62	3.51	3.75
Crude fat	1.21	1.18	1.22	1.15	1.18	1.20	1.17	1.21	1.18	1.15
Crude protein	14.98	13.23	13.45	13.67	12.58	13.34	13.89	13.67	12.34	13.34
Crude fiber	5.01	5.99	6.74	6.61	6.96	7.15	7.19	5.98	6.21	6.22
Reducing sugar	13.21	12.56	12.01	11.48	11.73	10.60	10.65	10.10	7.89	7.79
Total sugar	61.03	64.20	61.45	60.38	62.19	60.66	61.40	64.55	62.68	61.68

Table 3. Yields of solvent extracts of the red ginsengs stored for long periods

(Unit ; % of dry basis)

Extraction solvent \ Stored year	0	1	2	3	4	5	6	7	8	9
Water	41.85	41.72	40.19	40.81	40.60	40.12	42.08	40.39	42.14	40.81
50% ethyl alcohol	35.02	35.93	35.42	34.64	34.71	35.20	36.09	35.14	35.71	35.13
Ethyl acetate	1.14	1.20	1.21	1.25	1.30	1.29	1.33	1.31	1.38	1.42
Methylen chloride	0.85	0.90	0.93	0.96	0.94	0.98	1.06	1.03	1.16	1.23

Table 4. Saponin contents of the red ginsengs stored for long periods

(Unit ; % of dry basis)

Stored year	Crude saponin	Total saponin	Ginsenoside											PD*/PT**
			Ro	Ra	Rb_1	Rb_2	Rc	Rd	Re	Rf	Rg_1	Rg_2	Total	
0	4.37	3.13	0.07	0.06	0.55	0.27	0.30	0.15	0.29	0.09	0.69	0.06	2.53	1.14
1	4.26	3.01	0.07	0.06	0.53	0.25	0.28	0.15	0.28	0.08	0.68	0.05	2.43	1.17
2	4.29	3.04	0.07	0.05	0.53	0.27	0.29	0.16	0.28	0.08	0.69	0.05	2.47	1.18
3	4.39	3.08	0.07	0.06	0.55	0.26	0.29	0.16	0.29	0.09	0.68	0.06	2.51	1.18
4	4.32	2.98	0.07	0.06	0.53	0.26	0.29	0.15	0.28	0.08	0.69	0.05	2.46	1.17
5	4.37	3.12	0.08	0.06	0.54	0.27	0.30	0.16	0.29	0.09	0.68	0.06	2.53	1.19
6	4.18	2.81	0.07	0.05	0.55	0.27	0.30	0.15	0.29	0.09	0.66	0.05	2.48	1.21
7	4.16	2.84	0.08	0.06	0.52	0.26	0.29	0.15	0.28	0.09	0.64	0.05	2.42	1.21
8	4.14	2.73	0.08	0.06	0.51	0.25	0.27	0.14	0.27	0.08	0.62	0.05	2.34	1.22
9	4.29	2.96	0.08	0.06	0.53	0.26	0.28	0.14	0.28	0.08	0.65	0.05	2.41	1.20

* PD (Panaxadiol ginsenoside) : $Ra+Rb_1+Rb_2+Rc+Rd$

** PT (Panaxatriol ginsenoside) : $Re+Rf+Rg_1+Rg_2$

Table 5. Crude lipid content and percent composition of lipid components of the red ginsengs stored for long periods

Percent composition*	Stored year / Crude lipid content (%)	0	1	2	3	4	5	6	7	8	9
		1.21	1.18	1.22	1.15	1.18	1.20	1.17	1.21	1.18	1.15
Sterol esters & hydrocarbons		18.95	18.04	18.86	18.41	18.61	19.88	19.23	20.15	19.21	19.52
Triglycerides		31.07	30.55	30.77	31.33	31.04	29.58	28.88	27.98	28.26	26.68
Unidentified (I)		0.86	0.84	0.81	0.78	0.80	0.81	0.68	0.71	0.74	0.81
Unidentified (II)		1.00	1.01	1.12	1.06	1.01	1.03	1.11	1.53	1.56	1.65
Unidentified (III)		9.52	9.53	9.38	9.36	9.16	9.11	8.98	8.81	8.49	8.31
Free fatty acids		3.10	3.62	4.75	4.60	5.30	5.98	6.17	6.84	7.25	8.36
1.3-Diglycerides		9.14	8.40	8.21	8.34	8.11	7.82	6.74	6.58	6.39	5.99
Free sterols		9.78	10.03	10.21	10.61	10.26	10.32	10.96	11.01	11.19	11.85
1.2-Diglycerides		3.83	3.86	3.92	3.87	4.00	3.86	4.01	3.75	3.99	4.36
Polar lipids**		11.75	13.13	11.18	10.62	10.70	10.61	12.23	11.65	11.91	11.47

* Percent composition of crude lipids extracted by Soxhlet method with ethyl ether
** Contained glycolipids, phospholipids and monoglycerides

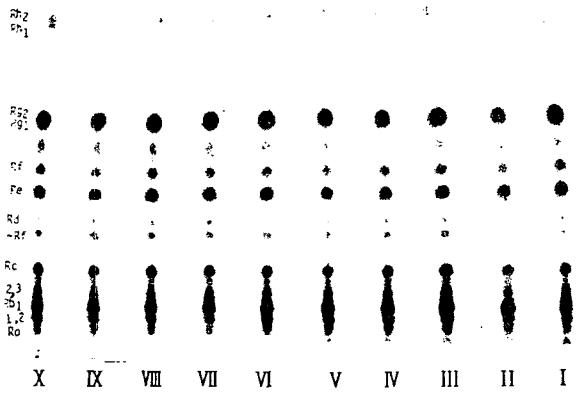

Fig. 1. Thin layer chromatograms of saponin components of the red ginsengs stored for long periods
* The stored periods of red ginseng samples were as follows : I. control, II. 1 year, III. 2 years, IV. 3 years, V. 4 years, VI. 5 years, VII. 6 years, VIII. 7 years, IX. 8 years, X. 9 years

Fig. 2. Thin layer chromatogram of crude lipids of the red ginsengs stored for long periods
*The stored periods of red ginseng samples were as follows : I. control, II. 1 year, III. 2 years, IV. 3 years, V. 4 years, VI. 5 years, VII. 6 years, VIII. 7 years, IX. 8 years, X. 9 years
**The lipid components were as follows : 1. sterol esters & hydrocarbons, 2. Triglycerides, 3. Unidentified (I), 4. Unidentified(II), 5. Unidentified(III), 6. Free fatty acids, 7. 1.3-diglycerides, 8. Free sterols, 9. 1.2-diglycerides, 10. Polar lipids (glycolipids, phospholipids and monoglycerides)

analyzed by TLC-scanner, respectively. Major lipids were triglycerides, sterol esters and hydrocarbons, 1.3-diglycerides, free sterols and unidentified component (III). Triglycerides and 1.3-diglycerides slightly decreased for long storage periods, while free fatty acids and free sterols slightly increased after 5 years of storage of red ginseng, but the compositions of lipids were considerably stable. This result seems to indicate that the hydrolysis of triglycerides and 1.3-diglycerides affected the increase of free fatty acids. And it is also supposed that glycolipids[21] such as sterol glucosides and esterified steryl glycosides in red ginseng were hydrolyzed to furnish free sterols and free fatty acids. But conspicuous changes were not observed in other lipid components. Lipid components were also stable during long storage, as was expected, when considering the manufacturing conditions and a slight variation of lipids in each raw red ginseng.

Fatty acid composition of crude lipids : Table 6 shows the percent compositions of fatty acids from crude lipids extracted with ethyl ether. Eighteen fatty acids[21] were identified from the crude lipids, the main fatty acids of which were linoleic ($C_{18:2}$), palmitic ($C_{16:0}$), oleic ($C_{18:1}$) and linolenic acid ($C_{18:3}$). As seen in Table 6, kinds and compositions of the fatty acids remained unchanged. Total unsaturated fatty acids and polyunsaturated fatty acids, which have double bonds and are subject to oxidation, remained almost unchanged in red ginsengs except in red ginseng stored for nine years.

Therefore, these data suggest that polyunsaturated

Table 6. Percent fatty acid composition of crude lipids of the red ginsengs stored for long periods

Fatty composition* \ Stored year	0	1	2	3	4	5	6	7	8	9
12 : 0	0.23	0.92	0.74	0.83	0.43	0.96	0.81	0.27	0.44	0.76
14 : 0	0.24	0.37	0.28	0.23	0.38	0.29	0.22	0.27	0.25	0.59
15 : 0	0.62	0.55	0.62	0.69	0.73	0.73	0.66	0.64	0.67	0.79
16 : 0	9.58	8.42	9.62	10.18	11.04	11.30	10.87	11.33	11.39	12.48
16 : 1	0.87	1.21	0.97	0.95	1.08	0.98	0.92	1.11	1.11	1.20
17 : 0	0.32	0.32	0.34	0.38	0.34	0.41	0.41	0.41	0.40	0.43
18 : 0	1.21	1.04	1.10	1.20	1.19	1.23	1.14	1.09	1.07	1.12
18 : 1	6.65	6.95	5.59	5.95	5.96	5.04	5.40	5.85	5.19	5.02
18 : 2	66.38	66.34	68.27	67.40	65.70	65.56	66.66	66.18	67.17	63.18
18 : 3	5.64	5.15	4.94	4.48	4.59	4.92	4.98	4.59	4.28	4.75
20 : 0	0.35	0.27	0.27	0.32	0.29	0.33	0.41	0.42	0.40	0.29
20 : 1	0.71	0.73	0.65	0.99	1.87	1.49	0.88	0.64	0.82	1.57
21 : 0	1.80	1.78	1.76	1.94	2.07	2.17	1.88	1.83	1.95	2.23
22 : 0	1.00	0.90	0.68	0.62	0.61	0.63	0.77	0.95	0.74	0.70
22 : 1	0.94	1.29	1.14	1.08	1.25	1.05	0.87	1.06	1.06	1.18
23 : 0	0.93	1.06	0.99	0.93	0.91	1.08	0.61	0.76	0.80	1.50
24 : 0	0.37	0.36	0.43	0.55	0.53	0.61	0.55	0.51	0.65	0.69
24 : 1	2.16	2.35	1.61	1.28	1.03	1.21	1.94	2.10	1.61	1.52
TSFA**	16.65	15.99	15.73	17.87	18.52	19.74	18.33	18.48	18.76	21.58
TUFA***	83.35	84.02	84.27	82.13	81.48	80.25	81.65	81.53	81.24	78.42
PUFA****	72.02	71.49	73.21	71.88	70.29	70.48	71.64	70.77	71.45	67.93

* Percent composition of crude lipids extracted by Soxhlet method with ethyl ether
** Total saturated fatty acids. *** Total unsaturated fatty acids. **** Polyunsaturated fatty acids (18 : 2+18 : 3)

and unsaturated fatty acids liable to oxidation are very stable during long storage because of the naturally occurred antioxidants[22,23] in ginseng and the specific antioxidants[3,14,24,25] formed by non-enzymatic browning reaction during manufacturing process and storage of red ginseng.

Color intensity : Red ginsengs were pulverized at 115 mesh to examine the color intensity (Table 7). The red filter value, "X", the green filter value, "Y", and the blue filter value, "Z" were measured by the CIE standard color system[13] and then converted into the yellow index, which revealed an increase of the intensity with storage time. By the Hunter color measure system[13], the "L" value, lightness, decreased, but both red intensity (the a value) and yellow intensity (the b value) increased.

Table 7. Measurement of color difference of the red ginsengs stored for long periods

Value \ Stored year / Manufactured year		0	1	2	3	4	5	6	7	8	9
		1986	1985	1984	1983	1982	1981	1980	1979	1978	1977
CIE value	X	40.36	35.62	34.60	30.20	33.80	33.49	35.39	41.62	34.26	24.80
	Y	40.12	34.86	33.45	28.84	32.61	32.32	34.16	40.71	34.02	23.00
	Z	27.25	22.04	20.46	17.16	20.41	19.85	20.27	24.54	18.49	13.05
Hunter value	L	63.34	59.04	57.81	53.64	57.10	56.85	58.43	63.80	58.33	47.95
	a	2.90	4.37	5.69	6.35	5.73	5.69	5.84	4.79	6.14	8.40
	b	18.84	19.22	19.49	18.60	18.80	19.10	20.36	21.86	22.03	17.44
Yellow Index*		56.23	63.27	67.06	70.49	65.83	67.04	69.24	66.48	74.80	77.42

* Yellow Index = 100 (1.28 × 1.0682)/Y
ASTM Committee : D-1925-70, D-1729-607, America Society for Testing & Materials, Philadelphia, U.S.A. (1975)

The brown color intensity of water extract and 50% ethanol extract solutions were measured at 490nm. As shwon in Table 7 and Table 8. brown color intensity increased with storage periods.

pH value : As shown in Table 9. the pH values of water extracts of red ginsengs decreased with storage time. indicating that it is related to the formation of various minor organic acids[26,27,31] and the increased amounts of non-enzymatic browning reaction products[28,29] formed progressively during the long storage periods.

Contents of free sugars : Free sugars are liable to react with amino acids to make Maillard browning reaction products. As shown in Table 10. maltose and total free sugar contents of red ginsengs remarkably decreased with storage time. Since reducing sugars[30-32] have been commonly known to be more reactive than non-reducing sugars. it is reasonable to presume that the decrease of maltose. a reducing sugar. is related to the browning reaction. or that it decomposed to yield two moles of glucose to react with amino acids.

Contents of free amino acids : Total free amino acids decreased conspicuously with storage time except those of red ginsengs stored for 5. 8 and 9 years (Table 11).

On the basis of preceding tables 7-10. it is suggested that the decrease of amino acids was done by the acceleration of the Maillard browning reaction[30-34]. Total free amino acids. arginine and threonine decreased conspicuously for the first 4 years of storage. but slightly after 5 years of storage. Accordingly. these findings led to the conclusion that the browning reaction was activated by free amino acids in the initial stage[30-34]. one to four years of storage. but in the final stage[30-34]. by interactions such as aldol type condensation or streker type reaction etc. of the intermediates of the browning reaction.

Free radical quenching activity : Antioxidant activity plays an important role in inhibiting lipid peroxidation. Free radical quenching activity[14,15] is the dominant theory for the anti-oxidant activity. The activities of red ginseng extracts were examined by the reduction of DPPH solution. Estimation of the activities of red ginsengs is shown in Table 12. The increase in the activities of 50% ethanol extract and water extract with storage time can be explained by the increased amounts of non-enzymatic browning reaction products[24,25,28-30] formed progressively during the long storage periods.

Antioxidant activity : To compare antioxidant activities. the fractions[3] extracted with ethyl ether and ethyl acetate were tested in relation to storage time by TBA values[3,16] of the linoleic acid-phosphate buffer substrate. The TBA

Table 8. Brown color intensity of extracts from the red ginsengs stored for long periods

Sample \ Stored year	0	1	2	3	4	5	6	7	8	9
Water extract solution*	0.129	0.150	0.210	0.240	0.214	0.240	0.228	0.261	0.284	0.331
50% EtOH extract solution*	0.051	0.062	0.113	0.165	0.124	0.134	0.134	0.158	0.213	0.205

* Each extract solution was prepared by extracting with fifity fold water or fifty fold 50% ethanol (v/w). and optical density was measured at 490 nm.

Table 9. pH value of water extracts from the red ginsengs stored for long periods

Sample \ Stored year	0	1	2	3	4	5	6	7	8	9
Water extract solution*	5.412	5.341	5.391	5.071	5.145	5.104	5.152	5.205	5.102	4.995

* Water extract solution was prepared by extracting with fifty fold water (v/w).

Table 10. Free sugar contents of the red ginsengs stored for long periods (Unit ; % of dry basis)

Free Sugars \ Stored year	0	1	2	3	4	5	6	7	8	9
Rhamnose	0.588	0.569	0.780	0.805	0.668	0.699	0.802	0.814	0.690	0.748
Fructose	0.364	0.447	0.468	0.447	0.401	0.428	0.334	0.396	0.462	0.471
Glucose	0.706	0.737	0.914	0.873	0.779	0.766	0.846	0.924	0.868	0.748
Sucrose	5.272	6.288	5.906	5.659	6.677	6.538	5.409	5.686	7.245	7.314
Maltose	9.755	9.932	9.850	9.352	9.883	8.702	8.663	7.964	6.410	5.727
Total	18.485	18.873	17.918	17.136	18.408	17.133	16.054	15.784	15.225	15.108

Table 11. Free amino acid contents of the red ginsengs stored for long periods

(Unit : % of dry basis)

Fatty amino acids \ Stored year	0	1	2	3	4	5	6	7	8	9
ASP	48.13	57.15	81.22	60.16	81.22	78.21	60.16	48.13	66.18	72.19
THR	51.14	45.12	42.11	36.10	36.10	42.11	45.12	36.10	39.10	36.10
SER	33.09	33.09	33.09	33.09	30.08	36.10	34.53	30.08	39.10	36.10
GLU	9.02	12.03	12.03	15.04	15.04	15.04	21.06	12.03	15.04	21.06
GLY	6.02	6.02	3.01	6.02	6.02	3.01	3.01	6.02	3.01	3.01
ALA	60.16	45.12	36.10	24.06	27.07	25.12	24.06	24.06	24.06	24.06
CYS	24.06	27.07	27.07	30.08	33.09	33.09	33.09	30.08	30.08	33.09
VAL	27.07	24.06	24.06	24.06	21.06	18.05	21.06	24.06	21.06	24.06
MET	27.07	27.07	30.08	30.08	30.08	24.06	30.08	30.08	30.08	36.10
ILE	18.05	21.06	24.06	27.07	24.06	24.06	24.06	27.07	30.08	33.09
LEU	12.03	12.03	9.02	9.02	9.02	6.02	9.02	9.02	9.02	12.03
PHE	66.18	60.16	96.26	102.27	99.26	126.34	138.37	123.33	126.34	132.35
LYS	75.20	78.21	87.23	90.24	81.22	99.26	87.23	105.28	99.26	120.32
HIS	21.06	18.25	15.04	15.04	15.04	18.05	15.24	15.04	15.04	15.04
ARG	899.39	601.60	541.44	436.16	427.14	478.27	403.07	469.25	475.26	538.43
Total	1,377.7	1,067.8	1,061.8	938.5	935.5	1,026.5	949.0	989.5	1,022.7	1,137.0

Table 12. The free radical quenching activity* of extracts from red ginsengs stored for long periods

Sample \ Stored year	0	1	2	3	4	5	6	7	8	9
50% EtOH extract	45.57	42.58	56.91	65.57	56.49	61.24	65.98	67.01	67.53	69.28
Water extract	44.33	42.06	58.35	63.09	57.42	57.94	59.90	59.79	61.44	70.21

*The free radical quenching activity was determined by percentage reduction of 1,1-diphenyl-2-picrylhydrazyl

values are frequently used as an index of the lipid peroxidation, such that a decrease in the TBA value corresponds to an increase in antioxidant activity. As shown in Fig. 3, antioxidant activity increased with time. According to the literature[14,22,23], antioxidant compounds have some relationships with pharmacological anti-aging action as shown by the inhibition of lipid peroxidation *in vivo*.

The inhibitory activities against lipid peroxidation in hepatic microsome : For the purpose of investigation of the anti-aging effect of Korean red ginseng in the biological system, we examined the inhibitory activities of red ginsengs with storage time against lipid peroxidation in hepatic microsome induced by enzymatic Paraquat/NADPH[18] and non-enzymatic Cu^{++}/H_2O_2[19] systems. As shown in Fig. 4, the inhibitory activities of red ginsengs against lipid peroxidation induced by Paraquat/NADPH system increase gradually with storage time. Fig. 5 also shows another inhibitory effect against lipid peroxidation induced by Cu^{++}/H_2O_2 system, which exhibited a similar trend to that of Paraquat/NADPH system. From the above facts, we conclude that the longer the storage period is, the stronger the inhibitory activity against lipid peroxidation in the same grade of red ginseng is. It, therefore, seems that red ginseng stored for long periods not only contribute to their quality stability, but also have a close relationship with their anti-aging effect. Further investigation, however, may be needed to study its mechanism of action as well as its action *in vivo*, and also to confirm its scientific efficacies.

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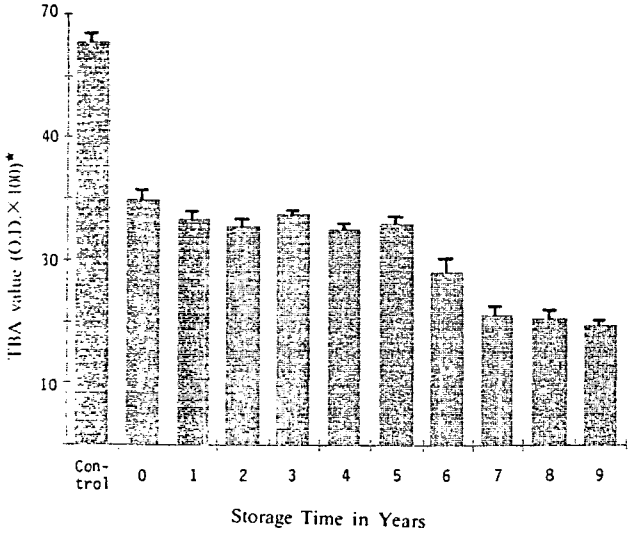


Fig. 3. Antioxidant activities of ethyl acetate fractions from red ginseng stored for long periods

* Ethyl acetate extracts from 1g of each red ginseng sample were added to linoleic acid-0.1M phosphate buffer substrate solutions. The TBA values of the mixtures were measured at 532 nm after shaking the mixture for 10hrs at 60°C and 100 rpm.

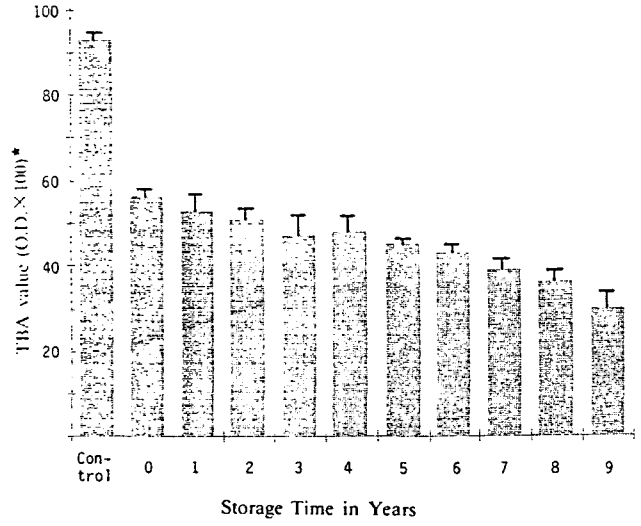
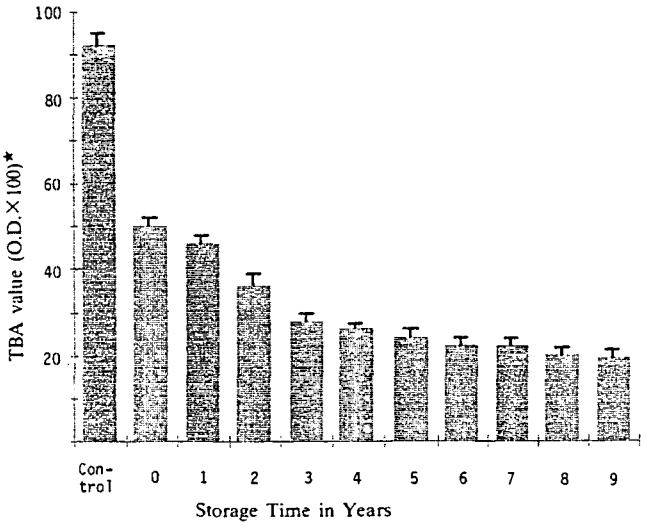


Fig. 4. The inhibitory effect of red ginseng against lipid hydroperoxide formation from hepatic microsomes induced by the enzymatic Paraquat / NADPH system.

* Microsomes (1mg protein / ml) were incubated for 1hr at 37°C in 50 mM Tris-HCl buffer (pH 7.5) with 10 μM Paraquat and 0.5 mM NADPH, along with 50 μg/ml of the ethyl acetate extract of red ginseng. The total volume of reaction mixture was 2.0 ml. The amount of MDA, lipid peroxide, was measured by the TBA method (A_{533}).



Fig, 5. The inhibitory effect of red ginseng against lipid hydroperoxide formation from hepatic microsomes induced non-enzymatic Cu^{++} / H_2O_2 system.

* Microsomes (1 mg protein / ml) were incubated for 1 hr at 37°C in 50 mM Tris-HCl buffer (pH 7.5) with 50μM $CuSO_4$ and 5 mM H_2O_2, along with 50μg/ml of ethyl acetate extract of red ginseng. The total volume of reaction mixture was 2.0 ml. The amount of MDA, lipid peroxide, was measured by the TBA method (A_{533}).

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H. Oura : Did you compare the antioxidant activity of red ginseng with that of white ginseng ?

K. J. Choi : I compared and published the results in the other paper. I am sure that red ginseng is more effective than white one. It is well known that the non-enzymatic browning reaction products in food and amino-carbonyl reaction model system show an antioxidant activity.

R. R. Bridges : Which components of ginseng possess major antioxidant activity ?

K. J. Choi : My current view is that phenolic compounds. including browning reaction products and maltol. play an important role of the activity in ginseng.

R. R. Bridges : Have you ever experimented on the mechanism of antioxidation of ginseng ?

K. J. Choi : Not yet. but the study is in progress in our laboratory.

장기저장 홍삼의 품질안정성과 항산화효과

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한국전매공사에서 년차별로 제조 포장된 홍삼시료를 실온조건(12-18°C. RH 55-68%)으로 1년에서 9년까지 장기저장하여 품질안정성 및 항산화효과를 조사하였다. 일반성분조성. 여러 용매별 추출물의 수율 및 사포닌의 TLC와 HPLC 패턴은 거의 변화가 없고 안정하였다. 지방질과 지방산에 대한 TLC 및 GLC 패턴도 거의 변화가 없었고. 특히 불포화도가 높아 산화되기 쉬운 linoleic acid (C18 : 2) 및 linolenic acid (C18 : 3)도 매우 안정하였다. 홍삼 갈색도는 저장기간이 경과되면서 뚜렷한 증가 경향을 보였고. 물 추출물의 pH 값은 다소 감소되었는데. 이러한 경향은 장기저장중 일어나는 갈색화반응 때문이다. 물 및 에탄올 추출물의 환원성 및 초산에칠 추출물의 항산화 효과도 증가되었는데 이와 같은 활성의 증가는 비효소적 갈색화반응 생성물들의 증가에 기인되는 것으로 생각된다. 그러므로 홍삼의 제조과정이나 장기 저장기간중 비효소적 갈색화 반응에 의해서 점진적으로 형성된 항산화 물질들은 전반적으로 홍삼의 품질을 안정시켜 줄 뿐만아니라 노화억제 약리효능과도 상관성이 매우 깊은 것으로 생각된다.